

AEROBIC AND ANAEROBIC SWIMMING SPEEDS OF SPERMATOZOA INVESTIGATED BY TWIN BEAM LASER VELOCIMETRY

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ABSTRACT The motility of bovine and ovine spermatozoa has been studied under aerobic and anaerobic conditions, using a dual beam laser velocimeter. Cells swimming under aerobic conditions were found to be characterized by a translational swimming speed and a rotation rate that were approximately double those of cells swimming in an anaerobic environment. Both types of spermatozoa have been found to exhibit a sudden coordinated transition between fast and slow swimming states when the available oxygen is exhausted. This transition from aerobic to anaerobic swimming states has also been shown to be reversible. Studies of the duration of aerobic motility using the same apparatus have shown that the cells have a constant motile efficiency over the temperature range 32°–42°C.

It is known that mammalian spermatozoa derive their motile energy from two metabolic pathways: fructolysis and respiration (1). Under normal conditions both of these pathways contribute to the overall energy requirements of the swimming cell, but the motility differs when one of the metabolic pathways is inhibited (2, 3). Direct measurement of the swimming speed distribution of spermatozoa under aerobic and anaerobic conditions and of the transition between these two states (when the available oxygen is exhausted) has not hitherto been feasible. We report here the results of experiments using a novel twin beam laser apparatus to study this transition for ovine and bovine spermatozoa. The data show that the transition is a remarkably rapid one that involves a reduction in both the rotational and the translational velocities of ~50%, and that this transition is reversible. We also report that the aerobic swimming speed, which varies with temperature, is proportional to the cell's oxygen consumption, implying a constant motile efficiency in the temperature range studied.

The twin beam laser apparatus used in these studies has been described elsewhere (4, 5). The important feature of this apparatus is that it permits the rapid determination of both the translational and the rotational velocity of large mammalian spermatozoa. Such cells are large compared with the wavelength of light, and the normal single beam laser Doppler technique is not adequate to yield these parameters unambiguously. In fact, the apparatus permits the extraction of various features of the velocity distribution of the population of cells, but in the following, only the mean speeds of the population are discussed. The apparatus

yields the mean rotation rate to better than $\pm 5\%$ in 2 min of data collection, and the mean translation rate to a similar precision in ~20 min.

In these experiments the sperm specimens to be examined by the twin beam velocimeter were diluted to an assay concentration of 2×10^7 cells/ml with "Caprogen," an egg-yolk citrate extender (5). They were then given an initial dose of oxygen, sufficient to maintain aerobic metabolism, and were enclosed in a sealed glass cuvette. By preventing gas flow to the sperm suspension the available oxygen was consumed by respiration in a time Δt determined by the initial oxygen concentration $[O_2]_i$, the final oxygen concentration $[O_2]_f$, the sperm concentration $[S]$, and the rate of oxygen consumption ZO_2 , i.e.,

$$\Delta t = \frac{[O_2]_i - [O_2]_f}{[S] ZO_2}, \quad (1)$$

where ZO_2 is the amount of O_2 in microliters consumed by 10^8 cells/h.

When the available oxygen has been exhausted, the cells are forced to switch to an anaerobic metabolism. By selecting an appropriate initial oxygen concentration, sperm swimming behavior under both aerobic and anaerobic metabolisms can be studied conveniently on the same sperm sample in the period before and the period after oxygen depletion.

The results of a typical experiment are shown in Fig. 1. Both the mean rotational and translational speeds drop to about half of their initial values after ~50 min in the sealed cuvette and remain constant for the next 100 min. The change in mean speed is particularly dramatic in the case

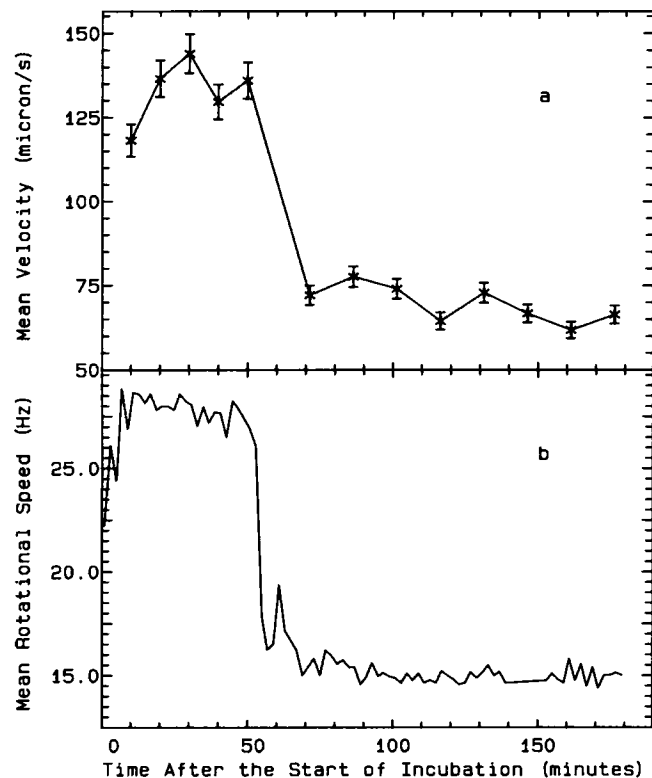


FIGURE 1 The change with time in (a) the mean translational speed and (b) the mean rotational speed of bovine spermatozoa. Sperm was diluted to an assay concentration of 2×10^7 cells/ml with an air-saturated "Caprogen" solution, enclosed in a sealed cuvette and incubated at 39°C . The sudden reduction in swimming speed after ~50-min incubation occurs when the dissolved oxygen has been exhausted by respiration.

of the rotational rate because of the better time resolution in the data (mean rotational speeds were determined every 2 min, mean translational speeds every 10 min). These experiments were performed at 39°C , and qualitatively similar results were obtained at other temperatures although the actual velocities are temperature dependent (5).

The fact that this sudden change in motility is associated with a switch from aerobic to anaerobic motility can be shown in various ways. First, Eq. 1 predicts a reciprocal dependence of the duration of aerobic motility on the sperm concentration. A graph of the duration of the initial fast motility pattern vs. reciprocal sperm concentration is shown in Fig. 2 and demonstrates clearly that the two are linearly related. While a similar graph could be expected if any of a number of metabolically important substances were to be depleted by sperm metabolism, the possibility that the transition from fast to slow motility was due to a factor other than oxygen loss was eliminated in these experiments by increasing the concentration of the sperm extender in step with the sperm concentration.

Confirmation of the oxygen loss hypothesis was also given by the agreement between the rate of oxygen consumption that can be inferred from the present experiment and that reported by other investigators. Taking the

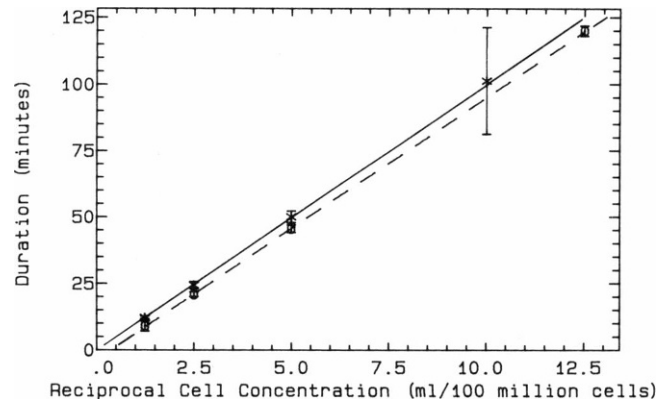


FIGURE 2 The duration of fast sperm motility as a function of reciprocal cell concentration for bovine (crosses) and ovine (circles) spermatozoa diluted with the "Caprogen" extender and incubated at 39°C . Error bars have been determined from repeated measurements on different ejaculates. Lines of best-fit to the bovine (solid lines) and ovine (dotted lines) data are also shown.

initial oxygen concentration of an air-saturated solution to be $4.4 \mu\text{l O}_2/\text{ml}$ at 37°C (7), applying a small correction for the different solubilities of oxygen at 37° and 39°C , and accepting Nevo's (6) value for the critical oxygen concentration at which aerobic motility ceases, the results presented in Fig. 2 imply a rate of oxygen consumption of $24.0 \mu\text{l}/10^8$ cells per h. This value is slightly higher than that of $21.0 \mu\text{l}/10^8$ cells per h reported by Lardy and Phillips (8) at 37°C , but a somewhat higher value is expected for cells moving at the faster speeds that are a consequence of the higher temperatures used here.

Eq. 1 for the duration of aerobic motility also predicts a linear dependence on the change in oxygen concentration. In another experiment initial oxygen concentrations were varied at a constant sperm concentration by saturating the sperm suspensions with air or nitrogen. The reduction in oxygen concentration in the partially nitrogen-saturated solution to 19% of the normal air-saturated value was matched by a reduction in the duration of fast motility to 17% of that under air saturation and provided another confirmation of the oxygen loss hypothesis.

Experiments with oxygen-saturated solutions were not reproducible, and this was traced to the toxic effect of hyperoxic conditions on the spermatozoa. This effect has been reported before (1, 9–11), and is thought to derive from the formation of hydrogen peroxide in solution.

The transition between aerobic and anaerobic motility is not restricted to bull spermatozoa, as Fig. 3 shows. Ram spermatozoa typically swim faster than bull spermatozoa at a given temperature (although the ratio of translational to rotational speeds is approximately the same, implying a similar swimming mechanism), and exhibit the same transition between states of high and low motility. In Fig. 3 it can be seen that the mean rotational rate of the population of cells drops by 50% in <2 min after 50-min incubation at 39°C . A 50% drop in the translational speed was observed simultaneously. The duration of aerobic motility for ovine

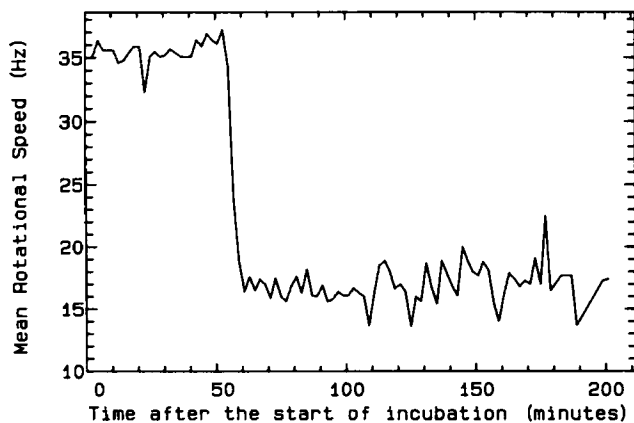


FIGURE 3 The change with time in the mean rotational speed of bovine spermatozoa. Sperm were diluted to an assay concentration of 2×10^7 cells/ml with an air-saturated "Caprogen" solution, enclosed in a sealed cuvette, and incubated at 39°C. The sudden reduction in swimming speed after ~50-min incubation occurs when the dissolved oxygen has been exhausted by respiration.

spermatozoa was also measured as a function of the cell concentration, and the results have been included in Fig. 2. These data yield a ZO_2 value of $27.1 \mu\text{l of O}_2/10^8$ cells per h, only slightly higher than the value for bull spermatozoa. This result is in agreement with the nearly equal respiratory rates previously reported (8).

The apparatus used in these experiments also permitted the reoxygenation of the sample after the transition between high and low motility states was complete. In this case the samples were withdrawn from the sealed cuvette after the transition, and agitated with air in a separate container. The sample was then reintroduced to the light scattering chamber and reassessed. This experiment was performed at three different cell concentrations and the results are shown in Table I.

Reoxygenation caused a return to the high motility state in each case. For concentrations of 20, 40, and 80 million

cells/ml the rotational speed recovered 96, 99, and 87% of the previous drop, respectively, and the mean translational rate recovered 92 and 78% of the drop for concentrations of 20 and 40 million cells/ml (the apparatus does not permit the reliable measurement of the translational swimming speed at concentrations of 80 million cells/ml [5]). The lack of complete recovery of motility in all cases is probably related to a slow aging effect, which we have also observed (5). It would therefore appear that bovine and ovine spermatozoa have the ability to switch rapidly between aerobic and anaerobic metabolism without cessation of motility. This ability would allow sperm striving to achieve internal fertilization to survive both when oxygen is scarce, as it is immediately after ejaculation, and when exogenous substrates are scarce.

The purpose of these experiments was to compare the swimming speeds of spermatozoa under aerobic and anaerobic conditions. These experiments also enabled the relationship between oxygen consumption and swimming speeds (that is the efficiency of aerobic motility) to be investigated. This was achieved by measuring the duration of aerobic motility at a given cell concentration as a function of the swimming speed of the cells. The swimming speed can be conveniently adjusted by varying the temperature of the sample. In these experiments mean rotational speeds were measured rather than mean translational speeds because of their greater ease of measurement. Previous observations (5) have shown that rotational and translational speeds maintain the same constant of proportionality (i.e., the same helical pitch length) over this temperature range. In the experiments performed in the temperature range 32°–42°C, over which the rotational speed increased by ~35%, it was found that the rate of oxygen consumption was proportional to the rotational speed and, therefore, to the translational speed, implying that the motile mechanism has a constant efficiency in this temperature range.

The existence of two swimming modes, aerobic and anaerobic, has proved to be useful in the *in vitro* assessment of sperm viability. The recovery of anaerobic motility by treated sperm samples provides an indication of the damage sustained by the site of anaerobic metabolism, the flagella, while the relative recovery of aerobic motility indicates the extent of damage to the mitochondria, the site of aerobic metabolism. In our studies of the recovery of motility by frozen-thawed bull sperm, we have found that aerobic motility declines to a markedly greater extent than anaerobic motility, suggesting that the mitochondria are more susceptible to damage by freeze-thawing than are the flagella.

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TABLE I

THE MOTILITY PARAMETERS OF OXYGENATED, DE-OXYGENATED, AND RE-OXYGENATED BULL SPERMATOZOA

Cell concentration	Oxygen status	Mean rotational frequency	Mean translational speed
$10^6/\text{ml}$		Hz	$\mu\text{m/s}$
20	Oxygenated	23.06 ± 0.37	126.0 ± 5.5
	De-oxygenated	13.15 ± 0.08	79.2 ± 3.5
	Re-oxygenated	22.20 ± 0.50	116.1 ± 5.1
40	Oxygenated	24.61 ± 0.24	130.6 ± 5.5
	De-oxygenated	13.23 ± 0.16	75.9 ± 3.2
	Re-oxygenated	24.40 ± 0.23	102.4 ± 5.6
80	Oxygenated	26.58 ± 0.55	—
	De-oxygenated	13.77 ± 0.31	—
	Re-oxygenated	23.18 ± 0.37	—

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